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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
Richard H. Tullis

Confirmation No.: 9155

Application No.: 08/078,768

Group Art Unit: 1631

Filing Date: June 16, 1993

Examiner: James Martinell

For: Oligonucleotide Therapeutic Agent And Methods Of Making Same

EXPRESS MAIL LABEL NO: EL 999261936 US
DATE OF DEPOSIT: April 15, 2004

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPEAL BRIEF TRANSMITTAL PURSUANT TO 37 CFR § 1.192

Transmitted herewith in triplicate is the APPEAL BRIEF in this application with respect to the Notice of Appeal received by The United States Patent and Trademark Office on **Herewith**.

☒ Applicant(s) has previously claimed small entity status under 37 CFR § 1.27 .

☐ Applicant(s) by its/their undersigned attorney, claims small entity status under 37 CFR § 1.27 as:

- ☐ an Independent Inventor
- ☐ a Small Business Concern
- ☐ a Nonprofit Organization.

☒ Petition is hereby made under 37 CFR § 1.136(a) (fees: 37 CFR § 1.17(a)(1)-(4) to extend the time for response to the Office Action of December 15, 2003 to and through April 15, 2004 comprising an extension of the shortened statutory period of one month(s).

	SMALL ENTITY		NOT SMALL ENTITY	
	RATE	FEE	RATE	FEE
☒ APPEAL BRIEF FEE	$165	$165.00	$330	$
☐ ONE MONTH EXTENSION OF TIME	$55	$	$110	$
☐ TWO MONTH EXTENSION OF TIME	$210	$	$420	$
☐ THREE MONTH EXTENSION OF TIME	$475	$	$950	$
☐ FOUR MONTH EXTENSION OF TIME	$740	$	$1480	$
☐ FIVE MONTH EXTENSION OF TIME	$1005	$	$2010	$
☐ LESS ANY EXTENSION FEE ALREADY PAID	minus	($)	minus	($)
TOTAL FEE DUE		$165.00		$0

☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or any future filing submitted to the U.S. Patent and Trademark Office in the above-identified application during the pendency of this application. The Commissioner is further authorized to charge any fees related to any such extension of time to Deposit Account 23-3050. This sheet is provided in duplicate.

☒ A check in the amount of **$165.00** is attached. Please charge any deficiency or credit any overpayment to Deposit Account No. 23-3050.

☐ Please charge Deposit Account No. 23-3050 in the amount of **$ .00**. This sheet is attached in duplicate.

☒ The Commissioner is hereby requested to grant an extension of time for the appropriate length of time, should one be necessary, in connection with this filing or any future filing submitted to the U.S. Patent and Trademark Office in the above-identified application during the pendency of this application. The Commissioner is further authorized to charge any fees related to any such extension of time to deposit account 23-3050. This sheet is provided in duplicate.

Date: April 15, 2004

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: **Richard H. Tullis** Confirmation No.: **9155**
Serial No.: **08/078,768** Group Art Unit: **1631**
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Sir:

APPELLANT'S BRIEF PURSUANT TO 37 C.F.R. § 1.192

This brief is being filed in support of Appellant's appeal from the rejections of claims 64-76 and 78-83 dated December 15, 2003. A Notice of Appeal and Request for Oral Hearing are filed herewith.

1. REAL PARTY IN INTEREST

Based on information supplied by Appellant and to the best of the undersigned's knowledge, the real party in interest in the above-identified patent application is ISIS Pharmaceuticals, Inc., a corporation of Delaware, which is the current assignee.

2. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences known to Appellant, Appellant's legal representative, or the assignee that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending Appeal.

3. STATUS OF CLAIMS

Claims 64-76 and 78-83 are pending in this patent application and are the subject of this Appeal. Claims 64-76 and 78-83 appear in Appendix A. The claims do not stand or fall together.

4. STATUS OF AMENDMENTS

No amendments to the claims were made in response to the Final Office Action dated September 10, 2002, the finality of which was withdrawn pursuant to 37 C.F.R. § 1.129. In response to the nonfinal Office Action dated June 17, 2003, Appellant filed a Reply pursuant to 37 C.F.R. § 1.111 wherein claim 75 was amended to delete the phrase "at a temperature between 0°C and 80°C" and claim 77 was canceled. The Final Office Action dated December 15, 2003 does not reflect entry of these claim amendments. Appellant is entitled to entry and consideration of the claim amendments pursuant to 37 C.F.R. § 1.129, and they are reflected in the presentation of claims in Appendix A. No amendments to the claims have been made subsequent to the Final Office Action dated December 15, 2003. Accordingly, claims 64-76 and 78-83 as amended in the response to the Office Action dated June 17, 2003 are involved in the present Appeal.

5. SUMMARY OF INVENTION

Appellant's invention relates to the use of oligonucleotides to specifically inhibit the expression of a target protein in a cell. The invention defined by the pending claims is the product of the surprising discovery made by the Appellant more than twenty years ago that oligonucleotides may be used to regulate protein synthesis in cells via hybridization to nucleic acids. Appellant's discovery provides for the systematic design and use of oligonucleotide agents to specifically block the translation of a target nucleic acid.

In seeking patent protection for his discovery, Appellant presents independent claims 64, 73, 75, 78, and 80. The invention, as recited in independent claim 64, relates to methods for selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids encoding both the target protein and other proteins without inhibiting the expression of other proteins. This can be accomplished by synthesizing an oligonucleotide having a base sequence substantially complementary to a subsequence of a messenger

ribonucleic acid encoding the target protein. The oligonucleotide is introduced into the cell where hybridization of the oligonucleotide to the subsequence of the messenger ribonucleic acid occurs to inhibit the expression of the target protein.

Independent claim 73 also recites methods for selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids encoding the target protein and other proteins without inhibiting the expression of the other proteins. According to the method of claim 73, one selects a synthetic oligonucleotide that has enhanced resistance against nuclease enzymes and a base sequence substantially complementary to a subsequence of a messenger ribonucleic acid of the cell encoding the target protein. The synthetic oligonucleotide is introduced into the cell and caused to hybridize with the messenger ribonucleic acid to inhibit the expression of the target protein.

The invention, as defined by claim 75, includes within its scope methods for selective inhibition of the expression of a target protein in a cell producing messenger ribonucleic acids encoding the target protein without inhibiting the expression of the other proteins. One selects a synthetic oligonucleotide having enhanced resistance against nuclease enzymes and a base sequence substantially complementary to a subsequence of a messenger ribonucleic acid of the cell encoding the target protein. The synthetic oligonucleotide is introduced into the cell where it hybridizes to the subsequence of messenger ribonucleic acid.

Claim 78 is directed to methods of selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acid encoding the target protein by selecting a base sequence substantially complementary to the messenger ribonucleic acid of the cell encoding the target protein, providing a synthetic oligonucleotide that is stabilized against *in vivo* degradative enzymes and having the selected base sequence, and introducing the synthetic oligonucleotide into the cell to hybridize to the subsequence of the messenger ribonucleic acid.

As defined by claim 80, Appellant's invention includes methods for selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids encoding the target protein by selecting a plurality of base sequences that are complementary to the messenger ribonucleic acid, providing a synthetic oligonucleotide corresponding to each of the base sequences, selecting a preferred synthetic oligonucleotide for inhibition of the target protein in a cell, and using the selected oligonucleotide to inhibit the target protein in cells.

Appellant's discovery has enabled the development of an array of therapeutic agents that address many previously unmet medical needs.

6. ISSUES

A. Whether or not the Examiner's factually unsupported surmise and speculation is adequate to refute the abundant evidence of record demonstrating that the specification and claims as originally filed enable a person of skill in the art to which the invention pertains to make and use the invention commensurate in scope with claims 64-76 and 78-83 without engaging in undue experimentation.

B. As to claim 71 alone, whether or not the Examiner has refuted the evidence of record demonstrating that claim 71 is patentably distinct over claim 1 of U.S. Patent No. 5,023,243.

7. GROUPING OF CLAIMS

Claims 64-70, 72, 80, 82, and 83 stand or fall together on appeal of issue A. Claims 71, 73-76, 78, 79, and 81 stand or fall together on appeal of issue A. Claims 64-70, 72, 82, and 83 do not stand or fall together on appeal of issue A with claims 71, 73-76, 78, 79, and 81 for the reasons set forth below.

Issue B applies only to claim 71.

8. ARGUMENT

A. The specification and claims as originally filed enable a person of skill in the art to which the invention pertains to make and use the invention without engaging in undue experimentation.

The rejection of claims 64-76 and 78-83 under 35 U.S.C. § 112, first paragraph for alleged lack of enablement is grounded in the Examiner's position that Appellant is required to have expressly taught known forms of stabilized oligonucleotides available at the time of filing in order to enable stabilized oligonucleotides other than phosphotriesters and to have successfully demonstrated inhibition of expression of a target protein using an antisense oligonucleotide *in vivo*. Appellant respectfully disagrees with the Examiner's position. As of the priority date, one of ordinary skill in the art was aware of the existence of stabilized forms

of oligonucleotides in addition to phosphotriesters and would have been guided by the disclosure of the application to the other stabilized oligonucleotides that are suitable, along with phosphotriester oligonucleotides, for *in vivo* use according to the claimed invention. Nothing more than routine experimentation was involved in determining which forms of stabilized oligonucleotides would have worked in the invention. Additionally, unmodified and modified oligonucleotides as used in the present invention are taken up by cells, are sufficiently stable to exert biological activity, and specifically hybridize to the target mRNA, as demonstrated in the examples provided in the specification. The cell culture models exemplified in the specification correlate to *in vivo* biological activity of the stabilized oligonucleotides, as substantiated by pre- and post-filing references. In short, the rejection of claims 64-76 and 78-83 under 35 U.S.C. § 112, first paragraph for alleged lack of enablement should be withdrawn because the evidence of record indicates that those skilled in the art as of October 23, 1981 having the benefit of Appellant's disclosure would have been able to practice the claimed invention without undue experimentation.

Preliminarily, Appellant respectfully asserts that claims 71, 73-76, 78, 79, and 81 are separately patentable from claims 64-70, 72, 80, 82, and 83 within the meaning of 37 C.F.R. § 1.192(c)(7). Claims 64-70, 72, 80, 82, and 83 are directed to methods of selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids. This is done by hybridizing a synthetic oligonucleotide having a nucleotide sequence substantially complementary to the subsequence of the messenger ribonucleic acid encoding the target protein to the subsequence of the messenger ribonucleic acid to specifically inhibit expression of the target protein. Claims 71, 73-76, 78, 79, and 81 recite methods for selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids. Expression of the target protein is inhibited by a synthetic oligonucleotide having a base sequence substantially complementary to a subsequence of the messenger ribonucleic acid wherein the synthetic oligonucleotide is stabilized to inhibit degradation by nucleases (claims 71, 78, 79, and 81) or has enhanced resistance against nuclease enzymes (claims 73-76).

Because claims 64-70, 72, 80, 82, and 83 do not require enhanced resistance to degradative enzymes or stabilization, in contrast to claims 71, 73-76, 78, 79, and 81, the enablement rejection of claims 64-76 and 78-83 based upon an asserted "problem with uptake and stability of unmodified oligonucleotides" (Office Action mailed June 17, 2003, page 10)

is limited to claims 64-70, 72, 80, 82, and 83. Therefore, claims 71, 73-76, 78, 79, and 81 are separately patentable from claims 64-70, 72, 80, 82, and 83.

The enablement requirement of 35 U.S.C. § 112, first paragraph, mandates that the specification teach those skilled in the art how to make and use the claimed invention without undue experimentation. *In re Wands*, 858 F.2d 731, 736-737, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) (citing *Minerals Separation, Ltd. v. Hyde*, 242 U.S. 261, 270 (1916)). The test of enablement is **not** whether **any** experimentation is necessary, but whether, if experimentation is necessary, it is **undue**. *In re Angstadt*, 537 F.2d 498, 504, 190 U.S.P.Q. 214, 219 (C.C.P.A. 1976). The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *Wands*, 858 F.2d at 737, 8 U.S.P.Q.2d at 1404.

The factors to be considered in determining whether any necessary experimentation is undue include:

i. the breadth of the claims;
ii. the nature of the invention;
iii. the state of the prior art;
iv. the level of one of ordinary skill;
v. the level of predictability in the art;
vi. the amount of direction provided by the inventor;
vii. the existence of working examples; and
viii. the quantity of experimentation needed to make or use the invention based on the content of the disclosure.

Id. (citing *In re Forman*, 230 U.S.P.Q. 546, 547 (Bd. Pat. App. & Int. 1986)). In order to make a rejection, the examiner has the burden to establish a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1561-62, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993). Assuming that sufficient reason for such doubt exists, a rejection for failure to teach how to make and/or use will be proper on that basis. *In re Marzocchi*, 439 F.2d 220, 223-24, 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971). The burden then shifts to the Appellant to provide persuasive arguments, supported by suitable proofs where necessary, that one skilled in the art would be able to make and use the claimed

invention using the application as a guide. *In re Brandstadter*, 484 F.2d 1395, 1407, 179 U.S.P.Q. 286, 294 (C.C.P.A. 1973).

The record demonstrates that, as of the time of filing, one of ordinary skill in the art would have been able to make and use the claimed invention using the application as a guide. No undue experimentation was required. Consideration of the *Wands* factors compels a finding of enablement: (1) the breadth of the solicited claims reasonably correlates to the enabled examples using phosphotriester oligonucleotides; (2) numerous modified oligonucleotides were known to one of skill in the art at the time of filing; (3) one of skill in the art of molecular biology in 1981 was highly sophisticated; (4) the specification as filed provided ample guidance, including examples, to one of skill in the art at the time of filing as to how to make and use the invention, which is all that is required for enablement; and (5) nothing more than routine experimentation was required to determine which modified oligonucleotides are most effective in the methods of the invention. Indeed, the record is replete with evidence – facts – underscoring the enablement of the present claims. The Examiner has met these facts only with surmise and skepticism.

It has been asserted that the present invention would not work *in vivo* using double-stranded oligonucleotides. (Office Action of June 17, 2003, page 3.) However, there has been no evidence adduced whatsoever for the assertion that a double-stranded oligonucleotide would not work in the claimed methods. This is an immutable requirement for maintenance of the rejection for alleged lack of enablement. The Examiner has merely stated that the term "hybridization" refers to the formation of a double-stranded nucleic acid by annealing of two single-stranded molecules and that the instant application does not disclose the formation of triplex DNA. (Office Action mailed June 17, 2003, page 3.) Appellant has not limited his invention to single-stranded oligonucleotides. Appellant asserts that double-stranded oligonucleotides work in the claimed methods, as supported, for example, by Mercola and Cohen (*Cancer Gene Therapy*, 2(1):47-59, 48-49 (1995)). Disclosure of the mechanism by which double-stranded oligonucleotides work in the invention is not required for enablement. Appellant need only have taught how to make and use the invention without undue experimentation. This Appellant has done.

1. Enablement does not require that Appellant expressly teach known forms of stabilized oligonucleotide available at the time of filing in order to enable stabilized oligonucleotides other than phosphotriesters.

The Examiner maintains that Appellant must have taught forms of stabilized oligonucleotides other than phosphotriesters to have enabled claims 71, 73-76, 78, 79, and 81, the claims requiring stabilization. Appellant disagrees.

Section 112 requires the specification to be enabling only to persons "skilled in the art to which it pertains, or with which it is most nearly connected." *DeGeorge v. Bernier*, 768 F.2d 1318, 1323, 226 U.S.P.Q. 758 (Fed. Cir. 1985). Thus, a patent need not teach, and preferably omits, what is known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 U.S.P.Q.2d 1331, 1332 (Fed. Cir. 1991); *Paperless Accounting, Inc. v. Bay Area Rapid Transit System*, 804, F.2d 859, 864, 231 U.S.P.Q. 649 (Fed. Cir. 1986) ("A patent applicant need not include in the specification that which is already known to and available to the public."); *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986). It has long been the law that a person skilled in the art is deemed to possess not only basic knowledge of the particular art, but also "the knowledge of where to search out information" for section 112 purposes. *In re Howarth*, 654 F.2d 103, 105, 210 U.S.P.Q. 689 (C.C.P.A. 1981).

Nowhere in the specification are the methods of the invention limited to phosphotriester-stabilized oligonucleotides. In fact, throughout the entirety of the specification, it is clearly stated that phosphotriester oligonucleotides are simply one representative example of the stabilized oligonucleotides that may be used in the methods of the invention. For example, the specification states at page 3, "[i]n a presently preferred embodiment of the invention, by way of example and not necessarily by way of limitation, a stabilized oligonucleotide, preferably in a phosphotriester form, is provided . . . " and at page 4, "[t]he preferred oligonucleotide . . . for increased stability, may be transformed to a more stable form, such as a phosphotriester form, to inhibit degradation during use." The application again states at page 5 that the oligonucleotide "can be transformed to a more stable form, such as a phosphotriester form, to inhibit degradation. . . ." Given the language of the specification including "such as," "preferred," and variations thereof, one of ordinary skill in the art readily understands that other forms of stabilized oligonucleotides were contemplated and equally useful in the methods of the invention.

That other forms of stabilized oligonucleotides were known in the art at the time of filing has been firmly established by the declarations of eminent scientists in the field, including Dr. Jerry L. Ruth (August 29, 1994 Declaration, Part 5A; April 14, 1995

Declaration, Part A), Dr. Dennis E. Schwartz (August 19, 1994 Declaration, Part 5A; April 14, 1995 Declaration Part A), and Dr. Stanley T. Crooke (Part 5). It is a fact that stabilized oligonucleotides suitable for use in the invention were known and were available to those of ordinary skill in the art in 1981. There is no countervailing evidence of record, none whatsoever.

References of record in this application also support this fact. For example, U.S. Patent No. 3,687,808 to Merigan *et al.* describes stabilized phosphorothioate oligonucleotides available as early as 1972. Miller *et al.* (*Biochemistry*, 13(24): 4887-4906 (1974) ("Miller 1974")) describe the stabilized alkylphosphotriester DNA analogs described in the application. Matzura and Eckstein (*Eur. J. Biochem.*, 3: 448-452 (1968)) describe the nuclease resistance of phosphorothioate oligonucleotides. Agarwal and Riftina (*Nuc. Acids Res.*, 6:9, 3009-3024 (1979)) show the synthesis of oligonucleotides containing methyl and phenylphosphonate linkages. DeClercq *et al.* (*Virology*, 42:421-428 (1970)) set forth the resistance of thiophosphate-substituted oligonucleotides to degradative enzymes. Miller *et al.* (*Biochem.*, 20(7): 1874-1880 (1981)) report a stabilized alkyl phosphonate DNA analog having activity *in vitro*. Holy ("Synthesis and Biological Activity of Some Analogues of Nucleic Acids Components," in PHOSPHORUS CHEMISTRY DIRECTED TOWARDS BIOLOGY, W.J. Stec, Ed., Pergamon Press, 53-64, 1980) describes modified nucleotide analogs having hydroxyl-containing aliphatic chains that are stable *in vivo* and display inhibitory and substrate activities. Harvey *et al.* (*Biochem.*, 12(2):208-214 (1973)) describe 5'-terminal alkyl phosphorothioate groups as protecting groups in oligonucleotide synthesis. Malkievicz *et al.* (*Czech. Chem. Commun.*, 38:2953-2961 (1973)) demonstrate the use of alkyl thioyl moieties as blocking groups in oligonucleotide synthesis.

Not only would one having ordinary skill in the art have readily understood that stabilized forms of oligonucleotides in addition to phosphotriesters were contemplated by the invention, but an artisan of ordinary skill also would have known of a number of available stabilized oligonucleotide forms as of the filing date. A routine literature search by an ordinarily skilled artisan at the time of the invention would have yielded a number of available stabilized oligonucleotides suitable for use in the invention, a sampling of which have been provided on the present record. (Crooke Declaration, Parts 3 and 5; April 14, 1995 Declaration of Dr. Schwartz, page 4; April 14, 1995 Declaration of Dr. Ruth, page 4.) As Appellant is not required to teach what is known in the art, his burden has been met.

The enablement requirement does not mandate that the Appellant have presented experiments with each of the available forms of stabilized oligonucleotides to demonstrate that they actually work in the invention. Rather, enablement requires only that Appellant have taught how to determine which stabilized oligonucleotides work in the invention without undue experimentation. Appellant has satisfied this burden by providing representative examples demonstrating his invention. One having ordinary skill in the art need only have substituted for the phosphotriester oligonucleotides of Appellant's examples other known forms of stabilized oligonucleotides to determine their efficacy in the invention. This would not have required undue experimentation on the part of an artisan of ordinary skill.

2. History has proven the naysayers of *in vivo* antisense technology to be incorrect; the present invention was complete and fully enabled in 1981.

The Examiner maintains that the methods of claim 64-76 and 78-83 would not work *in vivo* due to lack of cellular uptake, instability, and unpredictability of hybridization to the target mRNA. The Examiner has placed much reliance on the Gura (*Science*, 270: 575-577 (1995)), Rojanasakul (*Adv. Drug Delivery Revs.*, 18: 115-131 (1996)), and Hijiya (*PNAS USA*, 91:4499-4503 (1994)) articles allegedly to show that the present invention is not enabled for *in vivo* use. Appellant asserts that it is improper to base a conclusion of nonenablement upon these few references in view of their actual lack of significance and the numerous other references cited throughout the prosecution of the present application that contradict their allegations. The Declaration of Dr. Sidney M. Hecht, a premiere authority in the field of gene expression, supports this assertion.

Dr. Hecht first notes that any concerns raised by the Gura, Rojanasakul, and Hijiya references are directed to the clinical safety of *in vivo* use of antisense technology rather than at the efficacy of *in vivo* antisense methodologies. For example, Rojanasakul at page 118 queries "Can antisense work in living systems?" and responds by stating that while "there are studies which indicate the ***relative safety*** of antisense [oligonucleotides] *in vivo* . . . ***non-specific side effects*** of [antisense oligonucleotides] have also been reported in mice." Rojanasakul goes on to say that these safety concerns "do not diminish the potential use of [antisense oligonucleotides] *in vivo*, and there are few examples of successful *in vivo* treatment in the absence of specialized delivery systems." (*Id.*) Rojanasakul continues,

stating that "[c]onsidering the various obstacles that the antisense [oligonucleotides] must encounter prior to their action . . . ***the desired activity of [antisense oligonucleotides] is observed.***" (*Id.* (emphasis added)). Thus, Rojanasakul actually supports enablement of claims

Likewise, Dr. Hecht notes that Gura avers that "some experts in the field . . . argue that clinical trials have begun far too soon." (Gura at 575.) Dr. Hecht explains that such concerns regarding the clinical safety of antisense oligonucleotides were elicited by the side effects detected in some animal studies. For example, Gura describes one set of experiments in which lethality in monkeys administered a one-time, high-dose injection occurred as well as another set of experiments in which a transient decrease in two kinds of white blood cells and changes in heart rate and blood pressure resulted from the high dose administered. (*Id.* at 576.)

Similarly, the assertion that Hijiya characterizes the field of antisense as being "in its scientific infancy" is misplaced, according to Dr. Hecht. Hijiya makes clear that the unmodified and phosphorothioate-modified antisense oligonucleotides worked therein: "The experiments reported herein serve as a paradigm of [oligodeoxynucleotide]-based therapeutics for human malignancies." (Hijiya at 4503.) Hijiya reasons that, although *MYB* is an effective gene target of antisense oligonucleotides in human melanoma, "further development of the antisense strategy will be needed before the successful application of this technique ***in the clinic*** can be anticipated." (*Id.* (emphasis added).) Appellant likewise asserts that the Mercola reference describes several "signpost" studies in which a reduction in target protein was observed upon *in vivo* administration of antisense phosphorothioate-modified oligonucleotides complementary to the target genes in accordance with the presently claimed methods. (Mercola at 54-55.)

A demonstration of F.D.A. acceptable clinical safety is not required by the first paragraph of 35 U.S.C. § 112. Enablement does not require that the claimed invention satisfy the higher safety standards applied to drugs to be used in clinical trials. According to MPEP § 2107.03, "Office personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials.... [I]t is improper for office personnel to request evidence...regarding the degree of effectiveness [in humans] (underlining in original)." Enablement requires only that the application teach how to make and use the invention without undue experimentation. This requirement has been met: one having

ordinary skill in the art would be able to make and use the invention without undue experimentation using only the application as a guide.

Moreover, no drug is free of toxic effects. Fingl and Dixon (Chapter One, "General Principles", In THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 4th edition, L.S. Goodman and A. Gilman, Eds. (1970)). This fact has been known for many years, as substantiated by Dr. Hecht, and is as true today as it was when first presented in this textbook. For some authors to question the clinical safety of a new drug paradigm is not surprising. If raising such questions were to bar patentability of new drugs, there would be no new drugs. Accordingly, some toxic effects of antisense therapeutics are to be expected. Some expected toxic effects, however, are not an indication that antisense therapeutics do not work *in vivo*.

Indeed, Dr. Hecht attests that any concerns voiced by Gura, Rojanasakul, and Hijiya regarding the use of antisense technology *in vivo* have been proven to be wrong. The successes achieved in the field of antisense technology have been witnessed, thereby ratifying the views of proponents of antisense at the time of the invention and silencing, indeed converting, many critics to what is clearly the correct view: antisense works *in vivo* as taught by the present application.

A number of articles that corroborate the *in vivo* success of antisense technology have been cited during prosecution of the present application. For example, Mirabelli *et al.* (*Anti-Cancer Drug Design*, 6:647-661 (1991)) notes that antisense oligonucleotides have demonstrated activities against a broad array of targets, that "the therapeutic indexes of phosphorothioate oligonucleotides appear to be quite high," and that "certain phosphorothioates . . . are extremely well tolerated in animals." (Mirabelli at 651.) Mirabelli also provides evidence of successful *in vivo* trials of antisense oligonucleotides. (*See, e.g.*, Mirabelli at 653.)

Crooke (*Ann. Rev. Pharmacol. Toxicol.*, 32:329-376 (1992) ("Crooke 1992") corroborates the *in vivo* stability of antisense oligonucleotides, noting that nuclease activity of sera derived from different species varies, with human being the least active. (*See, e.g.*, Crooke 1992 at 337). Additionally, modified oligonucleotides enter cells at pharmacologically relevant concentrations. (*Id.* at 338-339.) *In vivo* pharmacokinetic studies reveal that antisense oligonucleotides are rapidly and broadly distributed following administration in mice, rabbits, and rats. (*Id.* at 342-343.) Toxicity studies reveal that phosphorothioate oligonucleotides, for example, have high therapeutic indices and exhibit

toxicity only at concentrations far in excess of concentrations at which therapeutic activity is observed. (*Id.* at 344; 346-347.) Indeed, Dr. Crooke has attested to these facts on the present record. (Crooke Declaration, Parts 6b and 6c).

Further confirmation of the enablement of Appellant's invention is found in Cossum (*J. Pharm. and Exp. Ther.*, 267(3):1181-1190 (1993)). That reference describes several *in vivo* studies in which phosphorothioate oligonucleotides were shown to be widely distributed following *in vivo* administration in nothing more than phosphate buffer at physiologic pH. (*See, e.g.,* Cossum at 1181-1182, 1186.) Additionally, Cossum acknowledges that the dosages at which non-antisense effects occur are significantly greater than those at which antisense effects are observed. (*Id.* at 1181.)

Stepkowski *et al.* (*J. Immunol.*, 153:5336-5346 (1994)) demonstrates specific inhibition of intercellular adhesion molecule-1 (ICAM-1) expression by antisense molecule IP-3082, thereby promoting heart allograft survival. (Stepkowski *et al.* at 5338.) Extension of *in vitro* studies to *in vivo* analyses confirmed the correlation between the efficacy of antisense technology in a Petri dish and in a living organism.

Appellant submits that, not only has the Examiner failed to consider references which run contrary to the few references upon which he relies to assert a lack of enablement, but the Examiner also is relying upon statements that have been proven false. Courts have long and uniformly held that the making of an invention in the face of skepticism by the scientific community is a hallmark of nonobviousness. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). Were the Examiner's views on the topic of enablement to prevail, the same skepticism which provides a powerful indication of nonobviousness would simultaneously eviscerate patentability under the enablement standard. This cannot be the law.

It is the Examiner's position that the time lapse between publication of the present application in 1983 and publication of results of success by skilled artisans in an active area of research weighs heavily against enablement of claims 64-76 and 78-83. The time lapse between the effective filing date of the present application and the numerous references cited in support of enablement of claims 64-76 and 78-83, however, has no bearing on the claim that the instant application provides sufficient guidance to one of skill in the art to practice the claimed invention as early as the effective filing date of the instant application. As Dr. Hecht attests, had the pharmaceutical industry in 1981 immediately applied its existing knowledge of medicinal chemistry and pharmacology to the teachings of Appellant, it would

have practiced the present invention. Various factors contributed to the lag, not the least of which included establishment, within an organization, of an internal "champion" for a new technology paradigm where the champion is willing to sponsor and defend reallocation of resources from existing programs to a new program. Additionally, once acceptance of the new paradigm is made, established pharmaceutical practice requires pharmacologists to perform substantial and numerous pre-clinical studies to determine the toxicological profile, pharmacokinetics, and pharmacodynamics of any potential drug. Thus, according to Dr. Hecht, it is not unexpected that the generation and reporting of pre-clinical and clinical studies by the pharmaceutical industry related to the efficacy of a potential drug does not immediately follow the publication of the first few positive *in vitro* results.

It has been observed by Fingl and Dixon that "[n]o drug is free of toxic effects." The authors further state, however, that "adverse effects do not arise solely because of the inherent toxicity of drugs and the limitations of the methods for early detection of this toxicity. *Many of the adverse effects could be avoided if drugs were used more carefully and more wisely*." (*Id.* at 26 (emphasis added).) Further, "[t]he development and evaluation of new drugs in the United States is rigidly controlled by federal regulation administered by the Food and Drug Administration. A new drug may not be marketed for general clinical use until it has been subjected to thorough clinical pharmacological studies and until 'substantial evidence' of its efficacy and safety have been obtained from adequate, well-controlled clinical trials conducted by qualified investigators." (*Id.* at 29.)

Since both positive and negative results must be included in data packages submitted to regulatory agencies, pre-clinical and clinical trials are not performed haphazardly with selective omission of negative results. In other words, slapdash animal studies are not performed for potential human therapeutic applications because all data collected is subjected to FDA scrutiny. Accordingly, every study is implemented pursuant to highly rigorous standards and carefully planned conditions. Animal tests suitable to regulatory agency submission require established animal colonies and adequate animal care facilities with appropriate veterinary oversight, the development of which is expensive and time-consuming. Accordingly, careful animal experiments do not yield large volumes of publications that appear in the literature quickly. They require systematic studies that may take years to accomplish. In other words, a significant delay in the reporting of pre-clinical or clinical results is entirely routine in the field of drug discovery and development.

Dr. Hecht also affirms that the numerous clinical investigations conducted on *in vivo* antisense methodologies underscore the belief of pharmaceutical companies and, hence, the skilled scientists that comprise them, in the efficacy of antisense technology *in vivo* as detailed by the present application. "Big" pharmaceutical companies became interested in antisense technology after the small pioneer companies confirmed its validity. For example, pioneer companies Hybridon Inc. and Isis Pharmaceuticals, Inc. were incorporated in 1989 for the purpose of developing antisense therapeutics. Gilead Sciences, Inc. formed in 1987 for the same purpose. Genta Inc. was established as a spin-off of Gen-Probe in 1988 with a business objective of developing antisense therapies initiated in Gen-Probe's diagnostic antisense studies. In the mid- and late 1990s, newcomers MethylGene Inc., Inex Pharmaceuticals Corp., and NeoPharma, to name only a few, joined the early-stage companies in exploiting the therapeutic aspects of antisense technology.

In contrast, as explained by Dr. Hecht, while contributing early-published papers regarding *in vitro* related research topics, individual academic researchers, who contribute much of the scientific literature, did not exploit and publish *in vivo* antisense technology. The reasons for this are varied. The exorbitant costs of animal studies, resulting from the necessity of numerous controls as well as the stringent regulations imposed by academic institutions and regulatory agencies, preclude most academic researchers from pursuing such studies absent industrial sponsorship. Additionally, the experiments conducted by most academicians are limited in scope by narrow, well-delineated areas of research interest. Accordingly, academic researchers do not perform isolated experiments that have no bearing on that research interest. Rather, academics are selective in choosing the focus of their experiments, limiting their experimental objectives to the particular area of research that fits into the grand scheme of the research to which their careers are dedicated, for which they have received institutional approval to study, and for which they have been granted funding.

Dr. Hecht summarizes that antisense technology was developed by small, early stage companies having limited resources. In view of the need of such companies to conserve their limited resources and the knowledge of such companies that a single poorly planned trial yielding a negative outcome could devastate an entire business venture, the pioneer companies in the antisense field had every incentive to perform animal trials carefully and systematically. They conducted animal trials in a highly methodical manner and at timepoints dictated by scientific and business judgment to advance to that phase in the

process of moving their drug candidates toward IND status. Pharmaceutical companies, including Isis Pharmaceuticals, Genta Inc., and Hybridon Inc. and their present or past large pharma partners including Novartis, Lilly, Abbott, Merck, Aventis, Amgen, Roche, and Boehringer Ingelheim, have invested huge amounts of time and money to verify the efficacy of antisense drugs in an effort to propel them through clinical phases and into the market. Given the enormous costs associated with drug development and marketing, pharmaceutical companies would not have invested so heavily in the development of antisense technologies if they believed antisense molecules would not work *in vivo*.

Indeed, clinical trials of antisense therapies have definitively established that antisense technology does work *in vivo* in accordance with the principles and guidance set forth in the present application. For example, the antisense drug fomivirsen (Vitravene™; Isis Pharmaceuticals, assignee of the present application) was approved by the FDA for the treatment of cytomegaloviral-induced retinitis in 1998. The Orange Book listing for fomivirsen identifies U.S. Patent Nos. 4,689,320, 5,264,423, 5,276,019, 5,442,049, and 5,595,978 as relating to that drug. The patents covering fomivirsen relate to compositions and methods for inhibiting propagation of a virus by employing an oligonucleotide hybridizable to a mRNA of the virus. In particular, U.S. Patent No. 5,595,078 claims methods for treating cytomegaloviral (CMV) retinitis by administering an oligonucleotide complementary to CMV mRNA to the subject requiring treatment. In practice, administration of the 21-mer oligonucleotide directed to the major immediate-early transcriptional unit of CMV is accomplished simply by contacting cells with the oligonucleotide in a pharmaceutically acceptable carrier or diluent such as saline. The FDA-approved mode of administration of fomivirsen for treatment of CMV is intravitreal injection, though any method that would place the relevant cells in contact with the antisense oligonucleotide would work. In short, fomivirsen works *in vivo* in accordance with principles of the presently claimed methods.

The Investigational New Drug Application (IND) for Fomivirsen was filed with the FDA in 1993, three years prior to publication of the Rojanasakul reference. Pre-clinical data was included as part of the IND. Thus, prior to the publication of the opinions of skeptics now relied upon by the Examiner, those skilled in the art already had obtained and submitted *in vivo* data to the FDA, data supporting results contravening that opined by the skeptics. Thus, prior to publication of the negative opinions of skeptics, those skilled in the art had

accomplished that which the skeptics opined would not work. In other words, those of skill in the art already were gathering *in vivo* data in support of their IND well before the publication of the opinions of antisense skeptics relied on by the Examiner.

Additional antisense therapies have proven successful in the clinic. Genta Inc.'s phase III clinical trials of the antisense drug Genasense™ also have demonstrated that antisense technology works *in vivo*, further refuting the Examiner's position that antisense is a highly unpredictable art. While the results of the Genasense™ phase III clinical trials were released publicly only in September 2003, clinical trials were begun years earlier. Indeed, the results of the very first phase I clinical trial of G3139 (oblimersen, Genasense™) in patients with non-Hodgkin's lymphoma (NHL) were reported at least as early as 1997 (Hayes, D.F., "Bcl-2 inhibition in the treatment of cancer: clinical studies with the Bcl-2 antisense oligonucleotide G3139", in BEYOND CHEMOTHERAPY, EMERGING TARGETED THERAPIES FOR THE TREATMENT OF CANCER, Symposium Proceedings, San Francisco, California, May 11, 2001, pages 12-18, the "Hayes reference", provided herewith as Exhibit A (citing Webb, *et al.*, Bcl-2 antisense therapy in patients with non-Hodgkin's lymphoma, THE LANCET, **1997 Apr 19**, 349(9059):1137-1141 (1997) ("the Webb reference," provided herewith as Exhibit B)). According to the Hayes reference, treatment with G3139 yielded one complete response, two minor responses, and nine patients with stable disease among 21 subjects:

> *Single-Agent G3139*
>
> The initial phase I trial of G3139 evaluated single-agent therapy in patients with relapsed NHL.[4,5] G3139 was administered for 14 days by continuous subcutaneous infusion as a single course of therapy. Only 1 course of therapy was planned, but responding patients could be considered for a second treatment course. A total of 21 patients were enrolled, and G3139 doses were escalated from 4.6 to 195.8 mg/m2/d. Three patients received 2 courses of therapy.
>
> Although all patients experienced inflammation at the infusion site, no significant systemic toxicities were noted until doses exceeded 110.4 mg/m2/d. The maximum tolerated dose was 147.2 mg/m2/d (4 mg/kg/d), and dose-limiting toxicities included thrombocytopenia, hypotension, fever, and asthenia. **Among the 21 patients, there were 1 complete response, 2 minor responses, and 9 patients with stable disease.** Correlative laboratory studies of tumor cells derived from peripheral blood, bone marrow, or lymph nodes indicated down-regulation of Bcl-2 protein in 7 of 16 samples.

> Overall, treatment with G3139 was found to be tolerable, with antitumor activity suggested in patients with relapsed NHL. Laboratory evaluation confirmed that therapy with G3139 could affect downregulation of Bcl-2 production at clinically achievable concentrations.
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>
> 4. Webb A., et al., *BCL-2 antisense therapy in patients with non-Hodgkin's lymphoma,* The Lancet, 1997; 349: 1137-1141.
>
> 5. Waters J.S., et al., *Phase I clinical and pharmacokinetic study of bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin's lymphoma.* J. Clin. Oncol. 2000; 18: 1812-1823.

Thus, even at the time that the critics of antisense therapies were formulating the criticisms now relied upon by the examiner, clinical results demonstrating successful treatment of patients with non-Hodgkin's lymphoma with antisense technology were being collected.

The mere fact that a few naysayers have predicted that methods such as those claimed would not work is of no relevance to the enablement of the instant claims because there is undisputed evidence on the present record that such predictions were incorrect. Some level of skepticism as to advances in science and technology has always been raised, and probably always will. In fact, the magnitude of such skepticism is arguably proportional to the magnitude of the advance. In the final analysis, it is not relevant whether skeptics exist but, rather, whether they were right.

Here, the evidence of record clearly demonstrates that the skeptics that the Examiner has identified were *not* right. The invention as set forth in the application and as presently claimed has been proven to work in the years following the effective filing date of the present application. Indeed, clinical trials of antisense therapies have established that antisense technology does work in accordance with the principles and guidance set forth in the present application, thereby dispelling the criticism of antisense skeptics including Gura and Rojanasakul.

In short, no further disclosure other than that made by Appellant in 1981 was necessary for those skilled in the art to practice the inventions as presently claimed without undue experimentation. This is the hallmark of enablement, and in no way is rebutted by the mere fact that there were those who doubted whether the underlying technology would ultimately be found to work. The methods that Appellant disclosed in 1981 have been

demonstrated to work repeatedly thereafter. No clearer case of satisfaction of the enablement requirement of 35 U.S.C. § 112 can be shown.

B. There is no obviousness-type double patenting.

Claim 71 stands rejected under the judicially created doctrine of obviousness-type double patenting as being allegedly unpatentable over claim 1 of U.S. Patent No. 5,023,243. Appellant traverses.

In determining whether a nonstatutory basis exists for a double patenting rejection, the issue is whether any claim in the application defines an invention that is merely an obvious variation of an invention claimed in the patent. When the claimed subject matter is patentably distinct from the subject matter claimed in a commonly owned patent, a double patenting rejection is improper. *Eli Lilly & Co. v. Barr Labs.*, Inc., 58 U.S.P.Q.2d 1865 (Fed. Cir. 2001). Any analysis employed in an obviousness-type double patenting rejection parallels the guidelines for analysis of a 35 U.S.C. § 103 obviousness determination (*In re Braat*, 19 U.S.P.Q.2d 1289 (Fed. Cir. 1991)); however, a double patenting rejection must rely on a comparison of only the claims. MPEP § 804, part III.

Claim 71 recites a method for selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids, the method comprising the steps of synthesizing an oligonucleotide having a base sequence substantially complementary to a subsequence of a messenger ribonucleic acid, wherein the subsequence encodes the target protein, introducing the stabilized oligonucleotide into the cell, and hybridizing the stabilized oligonucleotide to the subsequence of messenger ribonucleic acid to inhibit expression of the target protein.

In contrast, claim 1 of U.S. Patent No. 5,023,243 recites a method of selectively inhibiting *in vivo* synthesis of one or more specific targeted proteins comprising the steps of synthesizing an oligodeoxyribonucleotide having a nucleotide sequence substantially complementary to at least a portion of the base sequence of messenger ribonucleic acid coding for the targeted protein. In the claims of the '243 patent, at least a portion of the oligodeoxyribonucleotide is in the form of a phosphotriester to limit degradation *in vivo*. Claim 1 of the '243 patent calls for introducing the oligonucleotide into the cell and hybridizing the oligonucleotide to the subsequence of messenger ribonucleic acid to

substantially block translation of the base sequence and to inhibit synthesis of the targeted protein.

It is asserted that claim 1 of the '243 patent is a specific embodiment of claim 71, thereby rendering present claim 71 obvious. Appellant disagrees. The oligonucleotide of present claim 71 has a sequence substantially complementary to the *coding* portion of the target mRNA, whereas the sequence of the oligodeoxyribonucleotide of claim 1 of the '243 patent is substantially complementary to any region of the mRNA coding for the targeted protein. Because the portion of the target protein to which the oligonucleotide of claim 1 of the '243 patent is not limited to the coding region of the mRNA, that claim is not a specific embodiment of, and thus does not render obvious, claim 71 of the present application. No *prima facie* case of nonstatutory obviousness-type double patenting exists.

9. APPENDIX

A listing of the claims involved in the present Appeal, as amended by the response filed September 17, 2003, is provided in Appendix A.

Respectfully submitted,

Date: April 15, 2004

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: **Richard H. Tullis** Confirmation No.: **9155**
Serial No.: **08/078,768** Group Art Unit: 1631
Filing Date: **June 16, 1993** Examiner: **James Martinell**
For: **Oligonucleotide Therapeutic Agent And Methods Of Making Same**

EXPRESS MAIL LABEL NO.: US
DATE OF DEPOSIT: April 15, 2004

Mail Stop Appeal-Brief Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

APPENDIX A TO APPELLANT'S BRIEF

The following constitutes a complete listing of the claims on appeal. The claims do not stand or fall together. The Amendments of September 17, 2003 in response to the Office Action of June 17, 2003 are included.

64. A method of selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids encoding the target protein and other proteins without inhibiting the expression of the other proteins, said method comprising the steps of:

(a) synthesizing an oligonucleotide having a base sequence substantially complementary to a subsequence of a messenger ribonucleic acid said subsequence coding for the target protein,

(b) introducing the oligonucleotide into the cell; and,

(c) hybridizing the oligonucleotide to the subsequence of the messenger ribonucleic acid to inhibit the expression of the target protein.

65. The method of claim 64 wherein the entire sequence of the oligonucleotide is complementary to the subsequence of a messenger ribonucleic acid coding for the target protein.

66. The method of claim 64 wherein the oligonucleotide is at least 14 bases in length.

67. The method of claim 64 wherein the oligonucleotide is about 23 bases in length.

68. The method of claim 64 wherein the oligonucleotide is between 14 and 23 bases in length.

69. The method of claim 64 wherein the messenger ribonucleic acid is viral.

70. The method of claim 64 wherein the messenger ribonucleic acid encodes a hormone.

71. The method of claim 64 wherein the oligonucleotide is stabilized to inhibit degradation by nucleases.

72. The method of claim 64 wherein the oligonucleotide is an oligodeoxynucleotide.

73. A method of selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids encoding the target protein and other proteins without inhibiting the expression of the other proteins, said method comprising the steps of:

selecting a synthetic oligonucleotide that has enhanced resistance against nuclease enzymes and has a base sequence substantially complementary to a subsequence of a messenger ribonucleic acid of said cell, said subsequence coding for the target protein,

introducing said synthetic oligonucleotide into the cell, and

hybridizing said synthetic oligonucleotide to the subsequence of the messenger ribonucleic acid to inhibit the expression of the target protein.

74. The method of claim 73 wherein said synthetic oligonucleotide is between 14 and about 23 bases in length.

75. **(Previously presented in Amendment submitted September 17, 2003)** A method of selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids encoding the target protein and other proteins without inhibiting the expression of the other proteins, said method comprising the steps of:

selecting a synthetic oligonucleotide that has enhanced resistance against nuclease enzymes and has a base sequence substantially complementary to a subsequence of a messenger ribonucleic acid of said cell, said subsequence coding for the target protein, and

introducing said synthetic oligonucleotide into the cell to hybridize said synthetic oligonucleotide to the subsequence of the messenger ribonucleic acid.

76. The method of claim 75 wherein said synthetic oligonucleotide is between 14 and about 23 bases in length.

77. **(Canceled in Amendment submitted September 17, 2003)**

78. A method of selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acid encoding the target protein, said method comprising the steps of:

selecting a base sequence substantially complementary to said messenger ribonucleic acid of said cell coding for the target protein,

providing a synthetic oligonucleotide that is stabilized against *in vivo* degradative enzymes, said synthetic oligonucleotide having said selected base sequence, and

introducing said synthetic oligonucleotide into the cell whereby said synthetic stabilized oligonucleotide hybridizes to the subsequence of the messenger ribonucleic acid.

79. The method of claim 78 wherein said synthetic oligonucleotide is between 14 and about 23 bases in length.

80. A method of selectively inhibiting the expression of a target protein in a cell producing messenger ribonucleic acids encoding the target protein, said method comprising the steps of:

selecting a plurality of base sequences that are complementary to said messenger ribonucleic acid,

providing a synthetic oligonucleotide corresponding to each of said base sequences,

selecting a preferred one of said synthetic oligonucleotides for inhibition of the expression of said target protein in a cell, and

using said selected oligonucleotide for inhibition of said target protein in cells.

81. The method of claim 80 wherein said synthetic oligonucleotides are oligonucleotides stabilized against *in vivo* degradative enzymes.

82. The method of claim 80 wherein said selected synthetic oligonucleotide is between 14 and about 23 bases in length.

83. The method of claim 80 further comprising the step of synthesizing bulk amounts of said selected oligonucleotide for inhibition of said target protein *in vivo*.

1: Lancet. 1997 Apr 19;349(9059):1137-41. Related Articles, Links

ELSEVIER
FULL-TEXT ARTICLE

BCL-2 antisense therapy in patients with non-Hodgkin lymphoma.

Webb A, Cunningham D, Cotter F, Clarke PA, di Stefano F, Ross P, Corbo M, Dziewanowska Z.

Lymphoma Unit, Royal Marsden Hospital, Sutton, Surrey.

BACKGROUND: Overexpression of BCL-2 is common in non-Hodgkin lymphoma and leads to resistance to programmed cell death (apoptosis) and promotes tumorigenesis. Antisense oligonucleotides targeted at the open reading frame of the BCL-2 mRNA cause a specific down-regulation of BCL-2 expression which leads to increased apoptosis. Lymphoma grown in laboratory animals responds to BCL-2 antisense oligonucleotides with few toxic effects. We report the first study of BCL-2 antisense therapy in human beings. METHODS: A daily subcutaneous infusion of 18-base, fully phosporothioated antisense oligonucleotide was administered for 2 weeks to nine patients who had BCL-2-positive relapsed non-Hodgkin lymphoma. Toxicity was scored by the common toxicity criteria, and tumour response was assessed by computed tomography scan. Efficacy was also assessed by quantification of BCL-2 expression; BCL-2 protein levels were measured by flow cytometry in samples from patients. FINDINGS: During the course of the study, the daily dose of BCL-2 antisense was increased incrementally from 4.6 mg/m2 to 73.6 mg/m2. No treatment-related toxic effects occurred, apart from local inflammation at the infusion site. In two patients, computed tomography scans showed a reduction in tumour size (one minor, one complete response). In two patients, the number of circulating lymphoma cells decreased during treatment. In four patients, serum concentrations of lactate dehydrogenase fell, and in two of these patients symptoms improved. We were able to measure BCL-2 levels by flow cytometry in the samples of five patients, two of whom had reduced levels of BCL-2 protein. INTERPRETATION: In patients with relapsing non-Hodgkin lymphoma, BCL-2 antisense therapy led to an improvement in symptoms, objective biochemical and radiological evidence of tumour response, and down-regulation of the BCL-2 protein in some patients. Our findings are encouraging and warrant further investigations of BCL-2 antisense therapy in cancer treatment.

Publication Types:

- Clinical Trial
- Clinical Trial, Phase I

PMID: 9113013 [PubMed - indexed for MEDLINE]

BEYOND CHEMOTHERAPY

EMERGING TARGETED THERAPIES FOR THE TREATMENT OF CANCER

Proceedings

FROM A SATELLITE SYMPOSIUM HELD IN
SAN FRANCISCO, CALIFORNIA
MAY 11, 2001

BEYOND CHEMOTHERAPY

EMERGING TARGETED THERAPIES FOR THE TREATMENT OF CANCER

Proceedings

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Continuing Education Information

Target Audience

This continuing medical education program is intended for physicians and other health care professionals caring for patients with cancer.

Learning Objectives

Upon completion of this program, participants will be able to

- Describe the clinical development of epidermal growth factor receptor inhibitors, with a focus on their combined use with cytotoxic chemotherapy
- Discuss the clinical development of bcl-2 antisense technology in patients with advanced solid tumors
- Describe ongoing clinical and translational research utilizing farnesyl transferase inhibitors in aerodigestive tract cancers
- Identify therapeutic vaccine approaches that are currently being developed and evaluated in patients with cancer, with particular emphasis on the ALVAC canary pox vector

Sponsorship

This program is sponsored by Medical Education Resources.

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Faculty Disclosures

Waun Ki Hong has nothing to disclose.

Roy S. Herbst receives research support from AstraZeneca, Genentech, Imclone, Lilly, and TAP; is a consultant for AstraZeneca and Imclone; and is on the speakers' bureau for Aventis, Bristol-Myers Squibb, Glaxo SmithKline, and Lilly.

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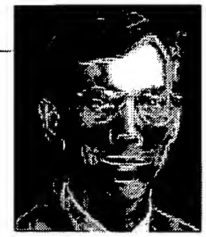
Neil L. Berinstein is an Aventis shareholder.

Bcl-2 inhibition in the treatment of cancer: clinical studies with the Bcl-2 antisense oligonucleotide G3139



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Overview of Bcl-2

Bcl-2 and its related family of proteins are important regulators of apoptosis, or programmed cell death.[1] Apoptosis appears to be a result of a balance of pro- and anti-apoptosis proteins. Key components of this family of proteins are the anti-apoptotic protein Bcl-2 and the pro-apoptotic protein Bax. Irreparable cell damage, such as DNA damage caused by exposure to cytotoxic agents or ionizing radiation, can initiate signals that begin a cascade of events leading to apoptosis. These events are initiated by mitochondrial release of cytochrome C, which results in activation of Apaf-1 and subsequent activation of caspases, which in turn induce apoptosis (Figure 1, panel A).

In the presence of an abundance of Bcl-2, the apoptotic pathway is blocked and the cell remains viable (Figure 1, panel B). Increased expression of Bcl-2, resulting in altered apoptotic regulation and accumulation of cells, is considered to be an important component of the malignant process of many tumors. Depletion of Bcl-2 permits apoptosis, perhaps in part by freeing Bax (Figure 1, panel C). In the normal cell environment, dimerization of Bcl-2 with Bax or related proteins prevents Bcl-2 from interacting with the pathway. Because of its importance in apoptotic regulation, Bcl-2 is a reasonable target for the development of novel therapeutic agents, such as antisense oligonucleotides.

Overexpression of the Bcl-2 protein is a common feature in many solid and hematologic malignancies (Figure 2). Of importance, high levels of Bcl-2 can confer substantial resistance to multiple classes of chemotherapeutic agents.[1,2] While cells that overexpress Bcl-2 do incur drug-induced damage, the otherwise expected initiation of the apoptotic process does not occur.

Bcl-2 Antisense Therapy with G3139

G3139 (oblimersen, Genasense™) is an 18-mer phosphorothioate oligonucleotide that targets Bcl-2 mRNA. G3139 anneals to mRNA, inhibiting its translation, resulting in decreased Bcl-2 protein synthesis. Phosphorothioate is used to stabilize the antisense oligonucleotide, preventing breakdown by RNA-ases. Preclinical data in Bcl-2–overexpressing human tumor xenograft models indicate that treatment with G3139 alone can inhibit tumor formation in a dose-

Figure 1. The relationship between Bcl-2 and apoptosis. Model of the apoptotic pathway (panel A) and blockade of apoptosis by interactions with Bcl-2 (panel B). Prevention of Bcl-2 interaction, through mechanisms such as Bcl-2 protein dimerization with Bax or inhibition of Bcl-2 protein translation via antisense oligonucleotides, reestablishes the apoptotic process (panel C).

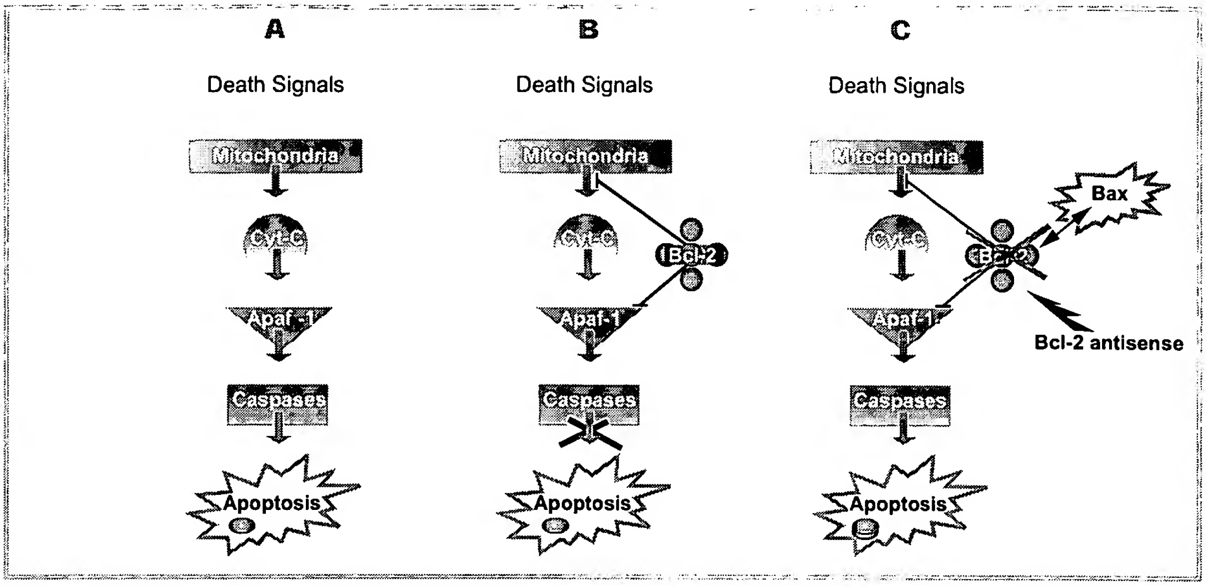
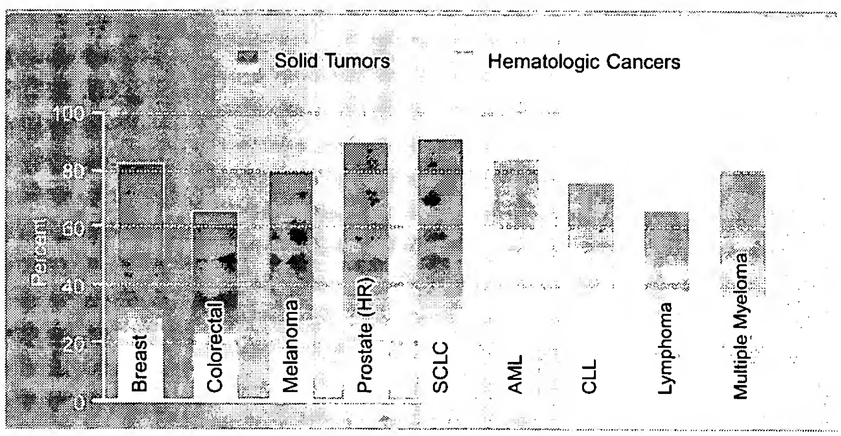


Figure 2. Overexpression of Bcl-2 in solid and hematologic malignancies



AML = acute myelogenous leukemia; CLL = chronic lymphocytic leukemia; HR = hormone-refractory; SCLC = small-cell lung cancer.

dependent manner.[3] Of particular interest, however, was the marked synergism observed when G3139 was administered in combination with subtherapeutic doses of docetaxel, paclitaxel, or cisplatin. In tumor xenograft–bearing animals so treated, complete tumor regression persisted for more than 5 months.

Several phase I studies with G3139 have recently been reported. Based on preclinical data, the very first trial of G3139 was conducted as a single-agent study in patients with non-Hodgkin's lymphoma (NHL).[4,5] In an effort to capi-

talize on potential synergistic effects, subsequent studies evaluated G3139 in combination with various chemotherapeutic agents (Table 1).[6-12] Current clinical trials are further evaluating the combination of G3139 with chemotherapy in a variety of tumor types (Table 2).

Single-Agent G3139

The initial phase I trial of G3139 evaluated single-agent therapy in patients with relapsed NHL.[4,5] G3139 was administered for 14 days by continuous subcutaneous infusion as a single course of therapy. Only 1 course of therapy was planned, but responding patients could be considered for a second treatment course. A total of 21 patients were enrolled, and G3139 doses were escalated from 4.6 to 195.8 mg/m²/d. Three patients received 2 courses of therapy.

Although all patients experienced inflammation at the infusion site, no significant systemic toxicities were noted until doses exceeded 110.4 mg/m²/d. The maximum tolerated dose was 147.2 mg/m²/d (4 mg/kg/d), and dose-limiting toxicities included thrombocytopenia, hypotension, fever, and asthenia. Among the 21 patients, there were 1 complete response, 2 minor responses, and 9 patients with stable disease. Correlative laboratory studies of tumor cells derived from peripheral blood, bone marrow, or lymph nodes indicated down-regulation of Bcl-2 protein in 7 of 16 samples.

Table 1. Early clinical trials of G3139 and chemotherapy in solid tumors

Tumor Type	Regimen	No. of Evaluable Patients	Grade 3/4 Toxicities
Various[6]	G3139 1-4 mg/kg/d CIV x 21 d Docetaxel 35 mg/m² d 8, 15, 22 q 28 d	14	Grade 3 thrombocytopenia in 1 patient
Breast and other solid tumors[7]	G3139 5-9 mg/kg/d CIV d 1-5, 12-13, 19-20 Docetaxel 35 mg/m² d 6, 14, 21 q 28 d	9	Grade 3 thrombocytopenia in 1 patient
HRPC[8]	G3139 5-7 mg/kg/d CIV d 1-5 Docetaxel 60-100 mg/m² d 6 q 21 d	18	Grade 4 neutropenia in 4 patients
Melanoma[9,10]	G3139 0.6-6.5 mg/kg/d CIV x 14 d Dacarbazine 800-1000 mg/m² q 21 d or G3139 5-9 mg/kg/d CIV x 5 d Dacarbazine 1000 mg/m² q 21 d	24	Grade 3 lymphopenia in 5 patients; grade 3 transaminase elevations in 4 patients in 14 d schedule
Colorectal[11]	G3139 3-7 mg/kg/d CIV d 1-8 Irinotecan 280-350 mg/m² d 6 q 21 d	19	Grade 3/4 diarrhea, grade 3 nausea and vomiting, and grade 4 neutropenia were dose-limiting
SCLC[12]	G3139 3 mg/kg/d CIV d 1-8 Paclitaxel 150-175 mg/m² over 3 h d 6 q 21 d	12	Pruritic rash necessitating study discontinuation observed in 1 patient

CIV = continuous intravenous infusion; HRPC = hormone-refractory prostate cancer; SCLC = small-cell lung cancer.

Overall, treatment with G3139 was found to be tolerable, with antitumor activity suggested in patients with relapsed NHL. Laboratory evaluation confirmed that therapy with G3139 could affect downregulation of Bcl-2 production at clinically achievable concentrations.

G3139 and Docetaxel

Based on preclinical data suggesting synergy between G3139 and chemotherapy, several phase I clinical trials evaluating G3139 combination regimens in a variety of tumor types have been initiated (see Table 1).

At the Lombardi Cancer Center in Washington DC, we evaluated the combination of G3139 and docetaxel in patients with advanced breast cancer and other solid tumors. In the first part of this phase I trial, escalating doses of G3139 were administered by continuous infusion on days 1 through 21, with docetaxel 35 mg/m^2 administered on days 8, 15, and 22 of a 28-day cycle.[6] The study enrolled patients with advanced breast cancer or other solid tumors that overexpressed Bcl-2. Overexpression of Bcl-2 was defined as at least 20% of tumor cells positive for overexpression by immunohistochemical assay. Prior taxane exposure was allowed.

Fourteen patients were enrolled over 4 dose levels of G3139, ranging from 1 to 4 mg/kg/d. Overall, the dose-limiting factor in this trial was fatigue, with 1 grade 3 thrombocytopenia and several episodes of grade 1 and 2 transaminitis.

Table 2. Clinical studies with G3139

Tumor Type	Study Type	Regimen	Population
Melanoma	Phase III	DTIC ± G3139	First-line, advanced disease
Multiple myeloma	Phase III	Dexamethasone ± G3139	Relapsed or refractory disease
CLL	Phase III	Fludarabine/Cyclophosphamide ± G3139	Relapsed or refractory disease
CLL	Phase I/II	G3139	Relapsed or refractory disease
AML	Phase II	Gemtuzumab ozogamicin (Mylotarg™) + G3139	Relapsed disease, elderly patients
AML/ALL*	Phase I	Fludarabine/cytarabine + G3139	Relapsed or refractory disease
Prostate*	Phase I/II	Docetaxel + G3139	Androgen-independent disease
Breast and other solid tumors*	Phase I	Docetaxel + G3139	Advanced disease
NSCLC	Phase II	Docetaxel + G3139	Second-line, advanced disease
Colorectal*	Phase I/II	Irinotecan + G3139	Metastatic or recurrent disease
SCLC*	Phase I/II	Paclitaxel + G3139	Recurrent disease
SCLC	Phase I	Carboplatin, etoposide + G3139	First-line, extensive stage

*Accrual complete.
ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; CLL = chronic lymphocytic leukemia; NSCLC = non–small-cell lung cancer; SCLC = small-cell lung cancer.

Pharmacokinetics studies indicated that with G3139 doses of 3-4 mg/kg/d, resulting plasma concentrations exceeded those previously noted in vitro to produce synergy with docetaxel.

In the second part of the study, shorter infusion schedules of G3139 were evaluated in an effort to decrease the incidence of fatigue and transaminase elevations.[7] Patients received G3139 on days 1-5, 12, 13, 19, and 20, with docetaxel 35 mg/m^2 on days 6, 14, and 21 of a 28-day cycle. Nine patients received G3139 in dose cohorts of 5, 7, and 9 mg/kg/d. The majority of toxicities were grade 1 or 2, with only 1 patient experiencing grade 3 thrombocytopenia. Overall, 2 patients had partial responses and 4 patients had disease stabilization.

Bcl-2 expression in circulating peripheral blood leukocytes (PBLs) was monitored by 2 methods: flow cytometry and Western blot. Results have been mixed, but they suggest that PBL Bcl-2 levels decrease during treatment and return to baseline upon discontinuation. Data from other phase I clinical trials have shown some decrease in tumor cell Bcl-2 expression.

The combination of G3139 and docetaxel has also been evaluated in a phase I trial in patients with hormone-refractory prostate cancer.[8] G3139 was administered in escalating doses of 5-7 mg/kg/d for 5 days followed by docetaxel 60-100 mg/m^2, with cycles repeated every 21 days. In the preliminary report of 18 patients, dose-limiting toxicity had not been reached, although 4 patients experienced uncomplicated grade 4 neutropenia. Flow cytometric and Western blot analyses indicated marked downregulation of Bcl-2 protein expression in peripheral blood mononuclear cells. Durable prostate-specific antigen (PSA) responses were seen in 7 of 12 patients without prior taxane exposure, with a 50-fold reduction in PSA and major objective responses in the liver and viscera. These preliminary safety and efficacy data support further investigation of the combination.

G3139 and Dacarbazine

The combination of G3139 and dacarbazine has been evaluated in patients with malignant melanoma (see Table 1, page 14). Initially, G3139 was administered intravenously or subcutaneously at doses of 0.6 to 6.5 mg/kg/d for 14 days in combination with dacarbazine 800-1,000 mg/m^2 per cycle.[9] Subsequently, G3139 was administered at doses of 5-9 mg/kg/d for 5 days in combination with dacarbazine 1,000 mg/m^2 per cycle.[10] The maximum tolerated dose of G3139 was 9 mg/kg/d administered by continuous IV infusion 5 days. In the 14-day schedule, toxicities were generally mild to moderate; however, 4 patients experienced grade 3 transaminase elevations and 5 patients had grade 3 lymphopenia. On the 5-day schedule, transient transaminase elevations occurred but were not dose-limiting. Laboratory studies demonstrated Bcl-2 downregulation and increased apoptosis after treatment.[10] Preliminary responses were encouraging, including several complete and partial responses, with demonstrated overall survival benefit.

G3139 and Irinotecan

The combination of G3139 and irinotecan has been evaluated in 19 patients with metastatic colorectal cancer (see Table 1, page 14).[11] G3139 was administered by continuous infusion on days 1-8 at doses of 3-7 mg/kg/d. Irinotecan was administered at doses of 280-350 mg/m^2 on day 6. Grade 3/4 diarrhea, grade 3 nausea and vomiting, and grade 4 neutropenia were dose-limiting with G3139 at a dose of 5 mg/kg/d in combination with irinotecan 350 mg/m^2. Laboratory studies confirmed Bcl-2 decreased protein expression in peripheral blood mononuclear cells. Among 9 patients previously untreated with irinotecan, there were 1 partial response and 2 patients with stable disease. Stable disease was also noted in 1 patient who had received prior irinotecan therapy.

G3139 and Paclitaxel

The combination of G3139 and paclitaxel has been evaluated in a phase I/II trial in patients with refractory small-cell lung cancer (SCLC) (see Table 1, page 14).[12] G3139 was administered by continuous infusion on days 1-8 at a dose of 3 mg/kg/d with paclitaxel 175 mg/m^2 on day 6. Dose-limiting hematologic toxicities were encountered in 2 of the first 3 patients treated, resulting in a dose decrease of paclitaxel to 150 mg/m^2 in subsequent patients. One patient developed a pruritic rash following therapy with G3139 and was removed from study; otherwise no toxicities greater than grade 2 were encountered. No objective responses were seen, although 2 of 12 patients (17%) achieved disease stabilization. At doses of G3139 of 3 mg/kg/d x 7 with paclitaxel 150 mg/m^2, treatment with the combination was considered tolerable.

Conclusions

Data from early clinical trials with G3139 indicate that therapy with this antisense oligonucleotide alone or in combination with chemotherapy is feasible, and early indications of efficacy are encouraging. The majority of systemic toxicities related to G3139 have been mild to moderate. However, the frequency of hepatic transaminase elevations in early trials using prolonged continuous infusions of G3139 was of some concern. With shortened G3139 infusion schedules, transaminase elevations appear to occur infrequently. Correlative laboratory studies have suggested adequate serum concentrations of G3139 can be achieved at clinically tolerable doses to effectively decrease Bcl-2 protein expression.

Preliminary data from several studies administering G3139 in combination with various chemotherapeutic agents, including docetaxel, dacarbazine, irinotecan, and paclitaxel, indicate that G3139 can be safely combined with chemotherapy. Preliminary reports of efficacy from these trials support the continued development of G3139 in several solid tumor types. Studies designed to determine the optimal dose and schedule of G3139 in combination with various chemotherapeutic agents are ongoing (see Table 2, page 15). Subsequent studies are planned to determine whether these combinations are superior to chemotherapy alone.

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